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Bioavailability/Toxicity of Iron from Aerobically Processed Organic Fertilizer

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BIOAVAILABILITY/TOXICITY OF IRON FROM AEROBICALLY PROCESSED ORGANIC FERTILIZER

by

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Approved:

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INTRODUCTION

For an undergraduate Honor's project, I worked under the direction of Dr. Jeffery Hall to determine the bioavailability and toxicity of iron from Milorganite® fertilizer, an aerobically processed organic fertilizer. The Milorganite® Company is a subsidiary of the Milwaukee Municipal Sewage District and produces a fertilizer of high iron content (approximately 5-7%). The high iron content has resulted in concern about the toxic potential, which until now was unknown. This thesis paper will explain iron chemistry, bioavailability, requirements, absorption, toxicity, and treatments, followed by a description of fertilizer types and contents. This will be followed by a description and reasoning for the dosing study and the results from our clinical and histological observations, serum biochemistry, and tissue mineral analysis. Finally, there will be a short summary and the conclusions we reached at the end of the study.

Iron is a transition metal located in the fourth period, Group VIII elements of the standard periodic table and has the atomic number 26. Transition metals found in the center of the periodic table, such as iron, have many important and unique properties. A very significant property that iron demonstrates is the ability to exist in both a di-and tri-valence state. Iron can easily convert from the ferrous (2+) state to the ferric (3+) state and vice versa because of the electron configuration of the valence shell. Two electrons are found in the outermost shell of iron. The two electrons allow iron to participate in ionic bonding by achieving

an oxidation state of 2+. By donating both electrons to form a bond, iron can achieve a complete octet and hence, become very stable. However, iron can also achieve a seemingly unstable oxidation state of 3+ by donating three electrons. While the ability to donate electrons leading to such an unstable state (less than an octet) may seem impossible, the outcome can be explained by observing iron's location in the periodic table. By donating three electrons, iron allows the d orbital to be only half full. Consequently, no electrons in the d orbital (which can contain a maximum of ten electrons) are paired, and a semi-stable configuration is obtained because all five remaining electrons spin in the same manner. The ferric state therefore allows for more versatility and stability.

The unique bonding ability of iron explains the diversity of iron containing compounds. Not only do ferrous and ferric compounds differ in bonding, but many other properties differ as well. An important difference in the chemical properties of iron compounds is solubility. Ferrous iron compounds are generally more water-soluble than ferric compounds. The difference in solubility of the compounds gives iron a diverse range of bioavailability because certain iron compounds will be soluble (and thus available) and others will not. However, it is important to keep in mind that iron compounds have the ability to convert between the ferric and ferrous states, thereby altering solubility as well.

Iron is a necessary element for mammals in many body functions. Heme groups (iron-containing porphyrin rings) in the hemoglobin of blood are responsible for the transport of oxygen throughout the body. In cellular respiration, the electron transport chain found in mitochondria is also composed

of heme groups that aid in the transfer of electrons. As electrons are passed between heme groups in the chain, energy is harvested and stored by forming ATP. Iron can also be found as a cofactor in an extensive list of enzymes necessary for a variety of body functions. For example, iron is essential for ATP production due to its central role in the electron transport chain. Peroxidase and catalase are also enzymes that utilize iron. Cytochrome enzyme systems are also examples of iron-containing proteins. Iron in the body is not only necessary to transport oxygen and produce ATP, but also is necessary to prevent anemia, fatigue, anorexia, stunted growth, and low resistance to infection, once again demonstrating that iron is a vital component to living systems (McKinley Health Center, 2001).

While iron is a necessary element, it is needed only in small quantities. Average iron consumption is 12-15 mg per day (Venugopal and Luckey, 1978). Once iron is consumed, concentrations within the body are regulated by absorption, not excretion. There are three mechanisms that regulate iron absorption. First, a dietary regulator acts such that increases in dietary iron are not continuously absorbed, due to the mucosal cells in the intestine having accumulated a manageable amount of iron and "blocking" additional uptake. Second, body stores regulate uptake so that as body iron stores fall, the mucosal cells are signaled to both moderately increase absorption and increase transport of iron from the mucosal cells into the blood. Finally, a eurythropoetic regulator by which, in response to anemia, the erythroid cells will signal the mucosa to increase iron absorption more significantly (Internet Pathology Lab, 2001).

Specific sites for iron absorption exist, however, competition by other elements for the active sites in the membrane can limit the amount of iron absorbed by the body. Iron can be more easily absorbed if a larger quantity is available. The bioavailability, nonetheless, depends on the form of the iron ingested. "The availability of Fe in a coordination complex rather than as an ion determines its absorption from the digestive tract and transfer across the mucosal cells" (Venugopal and Luckey 1978). Once soluble ferrous iron is ingested, it is absorbed in a carrier-mediated fashion in the mucosal cells of the intestine and converted to the ferric state.

Iron is stored by binding to the proteins ferritin or transferrin until the body indicates a need for iron in systemic circulation. The amount of ferritin in the blood is indicative of the amount of iron in the body because ferritin does not exist in the blood in large quantities. Once iron is needed, it is transported across the cell membrane via the transporter protein DMT 1 (the divalent metal transporter 1) into the plasma where it is bound to transferrin, the glycoprotein that allows iron to circulate in the bloodstream throughout the body.

In an iron overdose, the mucosal cells seem to become overloaded with iron, even though there is an adequate amount of iron in the bloodstream. Absorption appears to become passive and concentration-dependent, rather than carrier-mediated possibly due to direct cellular damage of the mucosal cells. Ferritin increases in the bloodstream indicating that an overdose has occurred. In extreme overdoses, the binding capacity of transferrin can also be surpassed resulting in free iron circulating in the plasma. Free iron circulation leads to iron

poisoning because free iron is highly reactive and exhibits tissue-damaging effects throughout the body (Greentree and Hall, 1995).

Free iron influences redox reactions with resultant lipid peroxidation and free radical formation (Massachusetts Poison Control System, 1997). Oxidation-reduction reactions release hydrogen ions that create an acidic environment within the blood and body thus disrupting the homeostatic pH of 7.4. Tissue effects are a direct result of both the peroxidative damage and the decrease in pH caused by the redox reactions. The effects of the redox reactions can primarily be observed in the gastrointestinal tract, liver, cardiovascular system, and brain because these organs have the greatest exposure to absorbed iron. Cells of these organs are damaged by free iron resulting in hepatic damage, myocardial failure, hemorrhagic necrosis in the lining of the stomach and small intestine, and seizures. The most significant effects can be observed in the cardiovascular system because excessive iron causes fatty necrosis of the myocardium, postarteriolar dilation, and increased capillary permeability. Consequently, reduced cardiac output, venous pooling, and diminished tissue perfusion occur (Greentree and Hall 1995).

Clinical iron toxicosis is generally divided into four stages. The first stage of toxicosis is characterized by vomiting, diarrhea (usually bloody), and abdominal pain. This stage begins acutely and can last for up to six hours postingestion. The second stage can vary in length, lasting up to 24 hours, depending on the amount of iron ingested. The second stage is characterized by an apparent stabilization or improvement in the syndrome. The apparent

recovery, however, is quite misleading. The most devastating effects are those of stages three and four. Stage three is the most serious because it involves multiple system organ failure. Symptoms of stage three are lethargy, recurrence of gastrointestinal signs, metabolic acidosis, liver necrosis, cardiovascular collapse and shock. Death can also result if proper treatment is not given. The fourth and last stage of iron poisoning is observed weeks after the initial effects when ulcerations heal causing gastrointestinal scarring and subsequent lumenal obstruction (Greentree and Hall, 1995; Massachusetts Poison Control Systems, 1997).

Treatment of iron poisoning begins with stabilization of vital signs, followed by decontamination, chelation, and supportive care. Degree of intervention should be based upon a physical examination of the patient, calculation of the amount of iron ingested, and results of diagnostic testing and radiographs (Greentree and Hall 1995). In cases where a large amount of iron has been consumed, blood tests for determining serum iron, serum iron binding capacity, and serum chemistry are recommended. Hydrogen peroxide (3%) should be given as an emetic. If induced vomiting is unsuccessful, a gastric lavage under anesthesia will be necessary. Possible consideration of an emergency gastrostomy is suggested if a large amount of iron was ingested. Restoring proper fluid, electrolyte, and acid-base balances is essential and intravenous fluids are necessary. Chelation using Deferoxamine is recommended for only the most severe cases. Patients should be monitored for signs of gastrointestinal obstruction following recovery. Should scarring occur, corrective intervention and

support management are necessary (Greentree and Hall, 1995).

Though toxic in large quantities, iron remains essential to life and can be consumed properly as part of a regular diet. There are two types of iron available for consumption. Heme iron is found only in meat, fish, and poultry and is absorbed easier than non-heme iron that is found primarily in fruits, vegetables, dried beans, nuts and grain products because heme iron has already been incorporated by the initial carrier into the necessary form for absorption (McKinley Health Center, 2001). Additional dietary iron is recommended for those people with an iron-deficient diet, pregnant women, and people who have experienced a high loss of blood. Dietary iron is available in vitamin supplements, which is the most common source of iron overdoses. Children and pets can accidentally consume a large quantity of dietary iron-containing vitamin supplements and experience acute iron toxicosis. There exists a possibility for iron to exist in bioavailable forms in processed organic fertilizer. Accidental ingestion of such a product could also lead to iron toxicosis, if the iron is highly bioavailable.

By definition, a fertilizer is a plant nutrient. Nitrogen, Phosphorus, and Potassium (N, P, K, respectively) are three elements required by all forms of plant life. Nitrogen promotes rapid, leafy green growth and builds plant materials, phosphorus helps the plant produce seeds and form roots, and potassium improves fruit quality and disease resistance. Therefore, most fertilizers used by farmers, ranchers, and gardeners today contain some amount of each of these elements. In fact, fertilizers can be distinguished by the ratio of NPK labeled on the bag. In addition to N, P, and K, there are a variety of other minerals that are

added for deficient soils in certain areas. The essential micronutrients are: Chlorine, Iron, Boron, Manganese, Zinc, Copper, Molybdenum, and Nickel, most of which are important in enzyme activities. Note that Iron is considered an important micronutrient, which in some areas may be lacking in the soil in a readily available form for plant use. Therefore, some fertilizers contain Iron in a form that plants can use but it is usually found only in parts per million to milligram concentrations, not as a percentage, like Milorganite®.

There are two types of fertilizer: man-made and organic. Man-made fertilizers are manufactured in water-soluble forms whereas organic fertilizers are of biological origin and contain organic material that is in the process of decomposing. Processed organic fertilizers have a biological origin but have already undergone decomposition and contain nutrients in a readily available form for plants. This processing of organic fertilizer can result in innumerable forms of iron salts and chelates. And, the form of the iron compounds within processed organic fertilizers may differ greatly between processing methods. The iron bioavailability therefore needed to be examined in order to determine the possibility of iron toxicosis if the product is accidentally ingested.

The main reason for conducting a dosing study is to investigate the potential adverse effects of fertilizer ingestion by humans (especially children) or copraphagic animals (especially dogs). Dogs, being copraphagic in nature, are attracted to fertilizers that are made of fecal material. The reason for conducting a dosing study in rats is because the bioavailability of the contents of the fertilizer are most readily studied in an animal model. Rats are good animal models for

bioavailability studies concerning dogs and humans because of their similar monogastric digestive system, metabolism, and methods of iron processing. Conducting a single study in this manner is also much simpler than conducting many studies in an attempt to determine the individual forms of the irons and their individual effects.

EXPERIMENTAL DESIGN

First, 36 rats were allowed to acclimate for one week before dosing and were weighed once at the beginning of the week and then again 24 hours before dosing. After acclimation, the rats were randomly assigned to one of six treatment groups to be dosed with 0, 100, 500, 1000, 1500, or 2000 milligrams of fertilizer per kilogram of body weight. The Milorganite® fertilizer product utilized was 6.8% iron. The rats were gavaged by dosing needle with ground fertilizer suspended in peanut oil (suspensions in water proved to be unsuccessful). During the acclimation and after dosing, the rats were given rat chow and water ad libitum and were monitored for one week for normal behavior, activity, eating, drinking, respiration, and feces production. After one week, the rats were euthanized in a CO_2 chamber. The chest cavity was opened and blood collected via cardiac puncture. Finally, tissues were collected for mineral analyses and pathologic evaluation. Mineral analyses were performed by Inductively Coupled Plasma/Mass Spectroscopy on the livers, kidneys, and small intestines (that had been washed in sterile water to remove the contents). Pathologic evaluations were performed on all of the major organ systems.

RESULTS

Clinically, we observed two animals with dosing complications. On day 1, these animals were removed from the study due to esophageal damaged sustained at dosing. All other rats appeared healthy and normal for the week following dosing. There were no differences in weight gain between the six treatment groups that were observed.

Histologically there were no lesions in any tissues identified in any of the treatment groups.

The serum iron and iron binding capacity were identical among all treatment groups as well. These two tests were performed to determine if iron was in excess in the body. If the serum iron is greater than the iron biding capacity (which is typically between 40 and 60% saturated) then there is free iron in circulation that can lead to iron toxicity. Our findings, however, maintained the correct ratios for all the treatment groups (serum iron was less than the iron biding capacity). A full serum chemistry panel including Total Protein, Albumen, Creatinine Phosphokinase, Alkaline Phosphatase, Sodium, Potassium, Calcium, Lactate Dehydrogenase, Creatinine, Blood Urea Nitrogen, Cholesterol, Total Bilirubin, and Aspartate Aminotransferase was analyzed and had no abnormalities or differences among the treatment groups.

Minimal differences in tissue mineral contents were identified. The mineral analyses of the kidney showed an increase in Nickel and Vanadium as dose increased. The small intestine analyses did not show any differences between the treatment groups for any minerals. Finally, the liver showed an unexplained statistical difference among treatments for Chromium, Nickel, and

Phosphorus. The differences are, however, illogical because the low and high dose groups showed similarities that were statistically different from the middle dose groups. Please see the attached tables for results of each tissue analyzed (Table 1-3). Iron, the primary mineral of interest had no differences among treatments for any of the tissues analyzed.

SUMMARY

To summarize, the high iron content in aerobically processed fertilizer proved to be not bioavailable as evidenced by the lack of increase in serum iron, iron binding capacity, and tissue iron content. Nickel and Vanadium, however, were bioavailable as observed by increasing concentrations in the kidney as dose increased. However, it must be remembered that this study was only a one-dose study and a long-term accumulation study is needed to investigate the severity and consequences of bioaccumulation of these minerals. Overall, this study noted no adverse effects due to the Milorganite® fertilizer product containing 6.8% iron.

In conclusion, Milorganite® fertilizer does not pose a health risk from the high iron content after acute ingestion. In addition, other minerals in Milorganite® did not result in adverse effects even though there seemed to be a dose response increase in Nickel and Vanadium in the kidney. Also, a study with multiple exposures is needed to assess the bioaccumulation of these minerals and determine the effects of multiple dosing on tissue accumulation of all of the minerals.

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TABLE 1

Milorganite Single Dose Study Rat Kidney Stats

Sample group	Control		100 mg/kg		500 mg/kg		1000 mg/kg		1500 mg/kg		2000 mg/kg	
Element	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
Ag	0.000	0.0000	0.000	0.0002	0.000	0.0004	0.000	0.0007	0.000	0.0000	0.000	0.0000
Al	0.067	0.0389	0.057	0.0340	0.094	0.0874	0.103	0.0248	0.071	0.0175	0.086	0.0558
As	0.344	0.0981	0.273	0.0177	0.320	0.0955	0.542	0.2735	0.345	0.0690	0.427	0.1045
B	0.232	0.0376	0.270	0.0718	0.261	0.0667	0.174	0.1193	0.247	0.0506	0.287	0.0779
Ba	0.006	0.0037	0.005	0.0024	0.006	0.0049	0.005	0.0033	0.003	0.0005	0.004	0.0010
Be	0.000	0.0002	0.000	0.0000	0.000	0.0002	0.000	0.0003	0.000	0.0000	0.000	0.0002
Ca	84.257	5.9659	89.409	17.3893	82.736	4.8375	85.280	3.1146	77.224	4.4053	78.569	5.5603
Cd	0.033	0.0443	0.016	0.0041	0.012	0.0024	0.014	0.0037	0.014	0.0023	0.016	0.0030
Co	0.152	0.0247	0.163	0.0140	0.170	0.0188	0.176	0.0193	0.159	0.0144	0.148	0.0100
Cr	0.174	0.0150	0.194	0.0151	0.186	0.0159	0.188	0.0065	0.179	0.0122	0.198	0.0255
Cu	6.995	1.5395	7.203	1.3919	7.458	1.6908	5.895	1.0079	6.139	0.8840	7.770	1.1850
Fe	50.921	9.0869	46.071	2.5490	50.186	10.1711	67.711	20.1378	52.789	4.4447	61.243	11.7491
K	2502.381	84.1871	2557.431	119.8337	2490.638	64.0854	2637.595	48.6252	2544.644	46.9198	2594.804	106.3562
Li	0.011	0.0033	0.010	0.0040	0.011	0.0028	0.010	0.0050	0.011	0.0029	0.012	0.0040
Mg	186.902	5.8493	190.792	8.9661	183.776	5.1545	193.613	7.0656	183.862	8.6350	190.789	5.8849
Mn	0.819	0.0445	0.825	0.0611	0.802	0.0579	0.851	0.0742	0.789	0.0581	0.820	0.0528
Mo	0.270	0.0240	0.274	0.0156	0.276	0.0102	0.279	0.0208	0.272	0.0329	0.288	0.0227
Na	1384.445	47.6541	1441.137	91.7632	1407.876	106.1304	1493.085	65.6371	1394.329	110.2174	1440.818	80.8921
Ni	0.004	0.0049	0.006	0.0024	0.005	0.0044	0.020	0.0150	0.008	0.0017	0.010	0.0057
P	2795.539	145.4647	2898.208	129.1308	2821.590	109.3744	3006.448	115.8223	2860.356	159.8496	2962.590	139.4154
Pb	0.005	0.0040	0.005	0.0017	0.004	0.0011	0.005	0.0026	0.003	0.0006	0.009	0.0064
Sb	0.001	0.0014	0.001	0.0013	0.001	0.0012	0.001	0.0016	0.001	0.0016	0.001	0.0016
Se	1.339	0.1399	1.271	0.1100	1.276	0.1039	1.466	0.1189	1.351	0.0724	1.381	0.0654
Si	16.466	1.6849	16.781	1.1494	16.946	1.2079	18.091	1.1999	17.045	1.3634	17.936	1.8509
Sn	0.003	0.0010	0.004	0.0017	0.003	0.0003	0.005	0.0029	0.004	0.0012	0.004	0.0012
Sr	0.026	0.0041	0.032	0.0082	0.027	0.0048	0.030	0.0081	0.025	0.0017	0.025	0.0038
Tl	0.017	0.0016	0.016	0.0013	0.014	0.0021	0.015	0.0023	0.015	0.0016	0.016	0.0016
V	0.024	0.0028	0.028	0.0040	0.032	0.0033	0.054	0.0233	0.041	0.0034	0.040	0.0033
Zn	20.480	1.2479	20.749	0.9845	20.174	0.6556	21.086	1.2428	19.815	0.8000	21.284	1.0326

TABLE 2

Milorganite Single Dose Study Rat Liver Stats

Sample group	Control		100 mg/kg		500 mg/kg		1000 mg/kg		1500 mg/kg		2000 mg/kg	
Element	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
Ag	0.001	0.0026	0.000	0.0005	0.000	0.0007	0.002	0.0027	0.000	0.0000	0.000	0.0005
Al	0.098	0.0065	0.140	0.0597	0.143	0.0442	0.157	0.0238	0.141	0.0394	0.139	0.0373
As	0.368	0.0279	0.334	0.0637	0.353	0.0865	0.449	0.2424	0.381	0.0762	0.332	0.0149
B	0.147	0.0223	0.145	0.0332	0.157	0.0289	0.124	0.0778	0.157	0.0322	0.172	0.0383
Ba	0.002	0.0007	0.005	0.0043	0.003	0.0010	0.003	0.0016	0.003	0.0011	0.004	0.0018
Be	0.000	0.0000	0.000	0.0000	0.000	0.0002	0.000	0.0000	0.000	0.0003	0.000	0.0003
Ca	56.112	3.3815	59.896	9.3481	57.822	3.1103	59.776	4.4725	65.540	9.4624	56.982	6.8562
Cd	0.011	0.0089	0.007	0.0009	0.006	0.0016	0.007	0.0010	0.007	0.0008	0.007	0.0010
Co	0.037	0.0049	0.039	0.0055	0.043	0.0140	0.042	0.0078	0.034	0.0086	0.035	0.0059
Cr	0.176	0.0122	0.172	0.0151	0.160	0.0116	0.145	0.0108	0.181	0.0109	0.171	0.0190
Cu	4.470	0.1640	4.425	0.4408	4.184	0.1406	4.778	0.6017	4.438	0.1952	4.371	0.3456
Fe	100.147	5.4267	102.515	5.7293	112.106	20.7134	112.104	28.7545	111.710	7.8615	92.228	13.7745
K	3507.296	114.8295	3463.598	68.8209	3473.393	118.3987	3619.955	129.3473	3488.297	92.7870	3461.037	165.4806
Li	0.006	0.0035	0.007	0.0034	0.007	0.0042	0.006	0.0041	0.006	0.0070	0.009	0.0069
Mg	222.186	3.5024	221.754	9.5358	219.682	6.4670	236.597	12.9617	222.572	4.2614	222.907	8.0088
Mn	2.673	0.1408	2.798	0.2367	2.630	0.0716	2.596	0.3558	2.880	0.1596	2.776	0.2139
Mo	0.711	0.0355	0.707	0.0246	0.685	0.0519	0.734	0.0957	0.731	0.0335	0.753	0.0800
Na	612.083	31.4696	595.004	64.6896	633.646	33.6183	663.472	100.5783	651.516	28.8900	641.996	62.7110
Ni	0.002	0.0008	0.005	0.0031	0.001	0.0008	0.004	0.0020	0.001	0.0013	0.002	0.0019
P	3898.115	60.4746	3744.661	122.4197	3633.079	143.1106	3863.544	249.8933	4005.239	93.1761	3654.985	205.9796
Pb	0.001	0.0006	0.002	0.0025	0.002	0.0019	0.003	0.0017	0.002	0.0029	0.001	0.0007
Sb	0.001	0.0024	0.001	0.0026	0.002	0.0026	0.002	0.0027	0.002	0.0029	0.002	0.0030
Se	1.094	0.0676	1.052	0.0485	1.071	0.0489	1.100	0.0998	1.059	0.0492	1.043	0.0849
Si	30.416	1.4936	30.997	2.3755	26.097	4.2300	24.888	1.4334	28.462	2.2619	28.013	4.6468
Sn	0.001	0.0017	0.002	0.0016	0.002	0.0010	0.004	0.0030	0.002	0.0024	0.002	0.0018
Sr	0.011	0.0015	0.015	0.0070	0.014	0.0026	0.017	0.0047	0.017	0.0046	0.014	0.0026
Tl	0.002	0.0002	0.002	0.0003	0.002	0.0002	0.002	0.0003	0.002	0.0003	0.002	0.0003
V	0.011	0.0008	0.012	0.0017	0.011	0.0013	0.021	0.0157	0.015	0.0015	0.014	0.0020
Zn	29.691	0.7828	29.0954	1.5788	28.218	1.0013	34.457	6.8559	31.202	1.5027	28.529	0.8762

TABLE 3

Milorganite Single Dose Study Rat Small Intestines Stats

Sample group	Control		100 mg/kg		500 mg/kg		1000 mg/kg		1500 mg/kg		2000 mg/kg	
Element	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
Ag	0.000	0.0002	0.000	0.0000	0.000	0.0003	0.000	0.0012	0.000	0.0000	0.000	0.0006
Al	0.395	0.1647	0.485	0.3203	0.555	0.3309	0.448	0.2559	0.415	0.2536	0.978	1.1552
As	0.060	0.0118	0.052	0.0063	0.072	0.0127	0.060	0.0161	0.065	0.0164	0.080	0.0309
B	0.153	0.0281	0.156	0.0321	0.162	0.0321	0.145	0.1058	0.163	0.0591	0.259	0.1307
Ba	0.038	0.0163	0.046	0.0374	0.053	0.0348	0.022	0.0286	0.032	0.0289	0.084	0.0956
Be	0.000	0.0002	0.000	0.0000	0.000	0.0000	0.000	0.0000	0.000	0.0000	0.000	0.0002
Ca	133.900	19.2475	157.788	48.3198	160.167	49.6909	142.992	53.3900	153.199	32.9687	187.261	81.3671
Cd	0.033	0.0642	0.004	0.0047	0.003	0.0008	0.015	0.0297	0.003	0.0010	0.004	0.0016
Co	0.015	0.0041	0.014	0.0024	0.018	0.0059	0.013	0.0061	0.017	0.0090	0.015	0.0041
Cr	0.139	0.0216	0.147	0.0315	0.181	0.0606	0.171	0.0550	0.203	0.0725	0.204	0.0917
Cu	1.536	0.1491	1.366	0.0945	1.507	0.2574	1.484	0.2784	1.565	0.2200	1.543	0.2920
Fe	12.497	2.9030	12.315	0.5494	14.165	2.6970	12.308	2.5983	13.276	2.1822	13.317	3.3582
K	2555.967	267.4238	2523.268	402.0085	2599.000	473.4581	2626.284	690.8316	2719.641	183.0196	2425.638	279.0190
Li	0.011	0.0050	0.010	0.0043	0.009	0.0032	0.011	0.0094	0.012	0.0062	0.018	0.0110
Mg	179.133	18.7092	170.535	10.8935	191.589	30.1184	174.447	49.9318	189.058	24.6540	180.072	28.9378
Mn	0.954	0.2541	1.010	0.1280	1.264	0.5241	1.097	0.3270	1.088	0.3323	1.431	0.8394
Mo	0.153	0.0239	0.132	0.0136	0.134	0.0260	0.127	0.0419	0.135	0.0230	0.147	0.0454
Na	1650.192	194.4901	1574.501	157.2891	1668.410	180.1173	1218.572	587.0473	1495.523	166.6660	1668.667	289.8123
Ni	0.022	0.0130	0.019	0.0109	0.037	0.0264	0.027	0.0183	0.035	0.0193	0.032	0.0087
P	2400.448	397.3695	2252.434	210.8535	2390.429	339.4894	2357.152	520.5701	2611.433	172.5872	2264.939	371.1685
Pb	0.002	0.0007	0.004	0.0029	0.004	0.0041	0.002	0.0012	0.002	0.0015	0.004	0.0025
Sb	0.001	0.0025	0.001	0.0018	0.001	0.0022	0.002	0.0035	0.002	0.0027	0.005	0.0052
Se	0.366	0.0304	0.344	0.0304	0.348	0.0598	0.312	0.0970	0.330	0.0201	0.320	0.0396
Si	17.712	1.1928	16.744	1.7986	22.492	4.3355	18.424	3.2271	21.967	1.7482	24.892	6.4100
Sn	0.003	0.0035	0.001	0.0011	0.002	0.0009	0.002	0.0015	0.003	0.0024	0.003	0.0018
Sr	0.070	0.0306	0.097	0.0582	0.092	0.0545	0.068	0.0503	0.069	0.0315	0.143	0.1125
Tl	0.002	0.0004	0.002	0.0002	0.002	0.0005	0.003	0.0010	0.003	0.0005	0.003	0.0012
V	0.018	0.0051	0.021	0.0061	0.027	0.0139	0.065	0.0465	0.034	0.0129	0.032	0.0166
Zn	20.408	1.9403	22.599	4.3103	26.605	4.7994	24.965	5.8584	27.100	4.3224	22.990	3.9378